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The Antimicrobial, Antioxidant and Antibacterial Effects of Seven Underexplored Medicinal Herbs were Evaluated

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Abstract - *Plants have a wealth of medicinal chemicals with vast potential in the pharmaceutical sector. The purpose of this research was to determine which phytochemicals were present in the seven medicinal plants chosen for this study, as well as to determine whether or not these plants have any antibacterial or antioxidant properties. Methods. The total phenolic and flavonoid contents, as well as the results of a standard phytochemical screen, were calculated. The antioxidant activity of plant extracts was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH), and nitric oxide (NO) radical scavenging assays. The broth microdilution technique was used to test the plant extracts for antibacterial activity. Phenols, flavonoids, and steroids were found in every plant extract analyzed by phytochemical methods. The extract of Psychotria peduncularis showed the highest total phenolic and flavonoid contents (5.57 • } 0.22 mg GAE/g and 1.38 • } 0.06 mg QE/g, respectively). The IC_{50} values for DPPH radical scavenging and NO radical scavenging ranged from 0.55 to 49.43 g/mL and 0.65 to 13.7 g/mL, respectively, indicating that all plant extracts exhibited extremely significant antioxidant activity. With MIC values ranging from 16 to 1024 g/mL, extracts of both Tristemma Mauritian rum and P. peduncularis showed potent antibacterial activity. Extracts of T. Mauritian rum were bactericidal against all species examined. There was substantial antifungal activity (MIC 64 g/mL) observed between Candida albicans and extracts of Alsophila Marianna and P. peduncular. Our research suggests that the screened extracts of medicinal plants might be employed as resources for the creation of novel medications, namely as antioxidant and antibacterial agents.*

Key Words: Microbial Pathogens, Medicinal Plants, Antioxidant, Antimicrobial, DPPH

1. INTRODUCTION

Antimicrobial resistance is a growing problem due to the creation and spread of drug-resistant bacteria that have developed novel resistance mechanisms [1]. Multi- and pan-resistant bacteria (also known as "superbugs") are rapidly spreading over the world, causing diseases that cannot be treated with conventional antimicrobial medications like antibiotics or antifungals [2]. There are no new antimicrobials under clinical development. 32 medicines targeting WHO priority diseases are now under clinical research, although only six of them may be considered novel. Moreover, a significant problem is still the limited availability of effective antimicrobials. Shortages of antibiotics and antifungals have a significant impact on healthcare systems worldwide, regardless of economic growth [3, 4]. Overproduction of reactive oxygen species (ROS) has also been linked to the onset of a wide range of chronic and degenerative conditions, including cancer, and respiratory, neurological, and gastrointestinal illnesses [4]. Antioxidants, which may be produced endogenously or supplied exogenously, have a finely tuned role in controlling ROS concentrations under physiological settings.

Antioxidant deficiency, in conjunction with starvation, may make people more susceptible to oxidative stress, which in turn raises the probability of cancer incidence [4]. Furthermore, persistent inflammation, as seen in COPD, IBD, neurological illnesses, cardiovascular disease, and even aging [5], may cause the antioxidant defense to become overwhelmed. Vitamin D, an antioxidant vitamin, plays a crucial role in controlling biochemical processes that ensure healthy organ function. Some clinical evidence shows that antioxidant supplementation may reduce endogenous antioxidant depletion and the resulting oxidative damage [6]. Since antibiotic-resistant microorganisms are on the rise, and since reactive oxygen species (ROS) are implicated in a wide range of chronic and degenerative human diseases, scientists have begun looking for plant-based antioxidants and bioactive compounds with novel mechanisms of action to combat pathogenic microbes [7, 8]. To make informed decisions on the usage of medicinal plants, one must have access to reliable scientific data and a familiarity with the plants' chemical constituents. Plants have medicinal properties owing to chemical molecules inside them [9]. The use of medicinal plants is crucial in the search for safer alternatives to manufactured pharmaceuticals [10, 11]. Both conventional and alternative medicine have long relied on

plants and other natural ingredients, which are now commonly employed in the industrial medication manufacturing process. According to credible scientific studies [12, 13], herbs account for almost 25% of all pharmaceuticals used around the globe.

Although these plants have long been used for therapeutic purposes, not much research has focused on the phytochemical components of these plants. Furthermore, the antioxidant and antibacterial effects of these therapeutic herbs are little researched. Therefore, the purpose of this research was to assess the antioxidant and antibacterial activity of extracts from these therapeutic plants, as well as their phytochemical contents.

2. MATERIALS AND METHODS

2.1.MicroorganismsandMedia.

Four fungal strains: *Candida albicans* (ATCC 90029), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), and *Candida tropicalis* (ATCC 750) were used. The bacterial spp. used were *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 25923), and *Enterobacter atherogenesis* (ATCC 13048), and three clinical isolates, namely, *Providencia Stuart*, *P. aeruginosa,* and *Vibrio cholerae* C06. The ATCC was a source for the fungi and bacteria, whereas the Pasteur Institute Yaound'e was the source for the clinical bacterium isolates (Cameroon). The bacterial activation and antimicrobial tests both made use of Mueller Hinton agar (MHA, Dominique Deutscher SAS) and Mueller Hinton broth (MHB, Dominique Dutscher SAS). Sabouraud Dextrose agar (SDA, Liofilchem) and Sabouraud Dextrose broth (SDB, Liofilchem) was used for the activation of yeasts and antimicrobial assays, respectively.

2.2.Plant Sample Collection.

Seven fresh plant specimens, including H. decumbens, L. macrocarpa, T. mauritianum, C. stelluliferum, A. manianna, C. bougheyanum, and P. peduncularis, were collected in September of 2016 from a variety of locations within the Tombel subdivision, which is situated in the southwestern region of Cameroon. At Cameroon's National Herbarium, the plants' provenance as native to the country was examined and confirmed. The voucher numbers that were allotted to the various plants are listed in Table 1, which may be seen here.

2.3.Preparation of Plant Extracts.

After being harvested, the plants were washed with water, and after that, they were laid out in the shade and left to dry at room temperature. After the plant samples had been dried, they were ground into powder, and then one hundred grams of that powder from each plant sample was macerated in eight hundred milliliters of methanol. After that, each sample was filtered using Whatman No. 1 filter paper, and the methanol was extracted from each filtrate by running it through a rotary evaporator (Buchi R-200) while the pressure was decreased. Finally, the samples were combined and analyzed. The extracts were kept frozen at a temperature of 4 degrees Celsius in anticipation of future studies.

2.4.Preliminary Phytochemical Screening.

We were able to determine whether or not certain chemicals were included in each plant extract by using a method that had been devised by Harbone (1984) [28]. These components comprised, amongst others, alkaloids, steroids, glycosides, flavonoids, tannins, saponins, and terpenoids. Both the total phenolic content (TPC) and the total flavonoid content were determined with the use of a technique that was established by Dzoyem and Eloff [29].

3. ANTIOXIDANT ASSAY

3.1. DPPH Radical Scavenging Assay.

Dzoyem and Eloff's [29] protocol was followed to conduct the DPPH test. Simply put, 100 L of each plant extract sample ranging in concentration from 12.5 to 200 g/mL was combined with 900 L of DPPH solution (0.2mM) produced in methanol. After 30 minutes of incubation at room temperature and in the dark, the spectrophotometer reading was taken at 517 nm to determine the concentration of the combination. Positive control was performed using ascorbic acid, a negative control was performed using methanol, and a blank was performed using extract without DPPH. Formula: %I = ((AbsorbanceControl AbsorbanceSample)/AbsorbanceControl) 100 indicates the percentage of inhibition of DPPH radical scavenging. By graphing percentages of inhibition against concentrations of each sample, the IC_{50} required to scavenge 50% of radicals from each plant extract was determined.

3.2. Hydroxyl Radical Scavenging Assay.

Each plant extract's ability to scavenge hydroxyl radicals was measured using a modified version of the Fenton process published by Sowndhararajan and Kang [30]. To recap, 1.5 mL of each plant extract at varying concentrations (12.5-200 g/mL) was combined with 90 L of FeCl3 (4 mM) and 60 L of 1, 10-phenanthroline in a volume of 1.5 mL. (1 mM). Then, 150 L of H2O2 (0.17 M) and 2.4 mL of phosphate buffer saline (0.2M pH 7.4) were added. After 10 minutes of standing at room temperature, the spectrophotometer was used to measure the reaction mixture's absorbance at 560 nm to determine the outcome of the reaction. A buffer solution served as a negative control, whereas ascorbic acid was employed as a positive one. As with the DPPH radical scavenging experiment, the

IC_{50} and percentage of hydroxyl radical inhibition were determined using the methods described above.

In a nutshell, each plant extract (8192 g/mL) was diluted 2-fold with MHB in a total amount of 100 L in a 96-well microplate. Plant extract concentrations varied from 4096 ng/mL to 2 ng/mL. Once the microplate had been incubated at 37°C for 24 hours (bacteria) or 48 hours (yeast), 100 L of inoculum (1.5 106 CFU/mL for bacteria and 1.5 104 CFU/mL for yeast) was added to each well. The conventional medications (ciprofloxacin or ketoconazole) were added to one set of wells, while the other set served as a negative control and included bacteria or fungus. Each well was filled with 40 L of INT solution (0.2 mg/mL), and the microplate was incubated at 37°C for 30 minutes. The yellow dye of INT is changed to a pink hue when it comes into contact with living bacteria or yeast. The MIC was determined by noting the concentration of extract needed to avoid a detectable color change in the medium. After incubation for the MIC test, wells that did not indicate growth were re-inoculated with 50 L of the test organism, and the resulting volume was added to 150 L of MHB (bacteria) or SDB to determine the MBC or MFC (yeast). The microplate was then kept in a 37°C incubator for 48 hours. The minimal bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC) were determined by testing a variety of extracts against various microbial populations. The experiment was run three times, each time with a duplicate set of results.

Table 1: The study's medicinal plants' defining features.

Scientific Name	Part Used	Traditional Used	Previous Pharmacological Studies	Isolated Phytochemical compound
H.Documents	Leave	Eye Infection sprain, female infertility, trypanosomiasssis, hernia, beriberi and gastralgia.	Not reported	Not reported
L.Macrocarpa	Fruit	Genital stimulants/depressants, aphrodisiac	Not reported	Not reported
T.Mauritianum	Leave	Wounds, cough, and premenstrual tension	Antisalmonellal and antioxidant	2,4-di-tert-butylphenol 2 ((octyloxy) carbonyl) benzoic acid and sitosterol
C.Stelluliferum	Whole Plant	Amnionitis affecting the newborn, polyhydramnios	Immunomodulatory	Tannins
A.Manniana	Leave seeds, Stem bark	Filariasis	Antioxidant	Flavonoids, quinones, tannins, terpenoids, and steroids
C.Bougheyanum	Whole Plant	Not reported	Acute and sub-chronic toxicity	Not reported
P.Peduncularis	Leave	Heart conditions [26] toothache, convulsion, yellow jaundice, stomachache,	Not reported	Not reported

4. RESULTS

Phytochemical Analysis: Seven medicinal plants' methanolic extracts were analyzed for their phytochemical content, and the findings are summarised in Table 2. All the plant extracts were found to have phenols, flavonoids, and steroids. Except for anthraquinone, every phytochemical was present in the L. macrocarpa extract. In addition, all plants contained saponins, except for A. manniana and P. peduncularis.

Total Phenolic and Flavonoid Contents.: Figure 1 displays the phenolic and flavonoid contents of a variety of medicinal plants. P. penduncularis and T. mauritianum extracts had the greatest total phenolic content (5.57 0.22 mg GAE/g and 4.92 0.55 mg GAE/g, respectively). Extracts of C. bougheyanum and H. decumbens, on the other hand, showed the lowest TPC (0.79 0.06 mg GAE/g and 0.48 0.05 mg GAE/g, respectively). TFC was greatest for L. macrocarpa (0.11 0.01 mg QE/g) and lowest for P. peducularis (1.38 0.06 mg QE/g). Similar to the A. manniana extract (0.39 0.04 mg QE/g), the TFC of the C. stelluliferum extract was also 0.36 0.02 mg QE/g.

Antioxidant Activity.: Tabulated in Table 2 are the results of DPPH, OH, and NO radical scavenging experiments used to assess the antioxidant properties of extracts from medicinal plants. The plant extracts had IC_{50} values between 0.55 and 49.43 g/mL for the DPPH and 0.65 and 13.7 g/mL

for the NO assays. The IC_{50} values of the P. peduncularis extract were comparable to ascorbic acid when tested using the DPPH and NO techniques.

Table-2: Seven medicinal plants' phytochemicals in methanol extracts were analysed qualitatively.

Phytochemical groups	Plant extracts						
	Hd	Lm	Tm	Cs	Am	Cb	Pp
Alkaloids	-	+	-	+	-	+	-
Phenols	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Saponins	+	+	+	+	-	+	-
Triterpenes	+	+	-	-	+	-	+
Steroids	+	+	+	+	+	+	+
Anthraquinone	-	-	+	-	+	-	-
Tannins	+	+	+	+	+	-	+

Note: +: the presence of phytochemicals, − : absence of phytochemicals, *Hd*: *H. decumbens, Lm*: *L. macrocarpa, Tm*: *T. mauritianum, Cs*: *C. stelluliferum, Am*: *A. manniana, Cb*: *C. bougheyanum, Pp*: *P. peduncularis.*

Table 3: IC_{50} (μg/mL) values of seven medicinal plant extracts against DPPH, OH, and NO radical scavenging.

	IC_{50} (μg/mL)		
	DPPH	OH	NO
H. decumbens	35.07 ± 0.55	123.59 • ± 0.23	10.44 • ± 0.36
L. macrocarpa	49.43 ± 0.06	>1000	0.78 • ± 0.00
T. mauritianum	25.88 • ± 0.54	169.82 • ± 0.30	13.7 ± 0.81
C. stelluliferum	58.88 ± 0.59	79.06 • ± 0.80	5.15 • ± 7.07
A. manianna	37.15 ± 0.86	153.46 • ± 1.94	7.34 • ± 0.13
C. bougheyanum	30.97 • ± 0.10	67.29 • ± 0.55	5.5 8 • ± 0.06
P. peduncularis	0.55 • ± 0.00	512.86 • ± 0.93	0.60 ± 0.00
Ascorbic acid	0.45 • ± 0.00	52.6 • ± 0.35	0.52 • ± 0.00

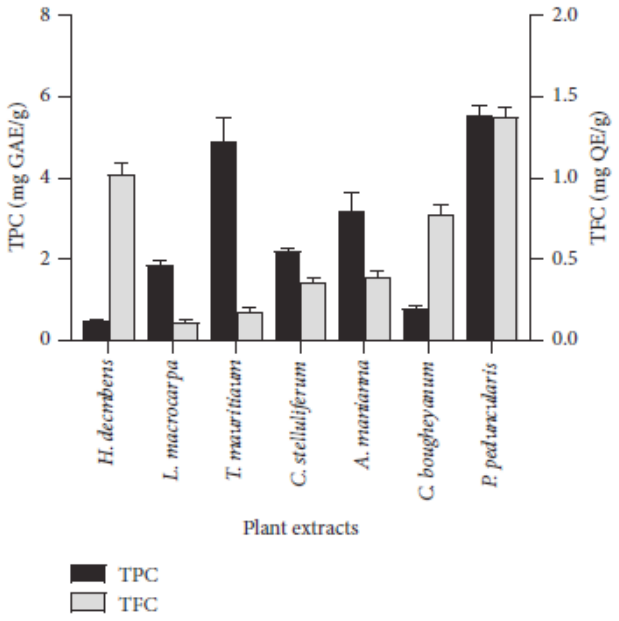

Figure 1: The total phenolic content (TPC) and total phenolic content (TFC) of seven extracts.

5. DISCUSSION

More and more nations are reporting that their citizens are using medicinal plants for their pharmacological effects. More than 25% of pharmaceuticals are produced from plants, according to the World Health Organization [12]. Phenols, flavonoids, and steroids were all identified by the phytochemical analysis as having key biological roles in the current investigation [28]. Anti-inflammatory, antispasmodic, antiulcer, antidepressant, antidiabetic, cytotoxic, antitumor, antibacterial, and antioxidant actions are only some of the many benefits associated with phenolic and flavonoid chemicals. Another benefit of therapeutic plant steroids is their ability to kill germs and insects [27]. Flavonoids, quinones, tannins, terpenoids, and steroids were all discovered in A. manniana by Ngbolua et al. [24], therefore our findings are consistent with their findings. In addition, Wickens and Burkill, using a similar financing model, demonstrated the presence of tannins in an extract of C. stelluliferum [21]. Saponins were found in every plant tested except for C. stelluliferum and P. peduncularis. As stated in [28], saponin-containing plant extracts have been used to treat inflammation, cerebrovascular and cardiovascular disorders, stomach ulcers, and UV damage. Saponins have also been utilized as adjuvants to improve the bioavailability of bioactive compounds and pharmaceuticals [29]. This study's plant extracts may have been used as a traditional medicine by the people of the Tombel subdivision since they contain certain phytochemical components.

Select therapeutic plants' total phenolic and flavonoid contents were also studied. The TPC and TFC levels were greatest in the P. penduncularis extracts. This plant may have enhanced biological capabilities due to its high concentrations of phenolic and flavonoid chemicals. No one test can definitively establish antioxidant activity [40]. DPPH, OH, and NO radical scavenging tests were used to ascertain the antioxidant activity of the medicinal plants under investigation. Very strong antioxidant activity is defined as an IC_{50} value of 50 g/mL or less, strong antioxidant activity as 50 g/mL to 100 g/mL, moderate antioxidant activity as 100 g/mL to 150 g/mL, and weak antioxidant activity as 150 g/mL or more [41]. This data demonstrates that all plant extracts tested had high levels of antioxidant DPPH and NOradical scavenging activity. Further, IC_{50} values of 79.06 g/mL and 67.29 g/mL were found for C. stelluliferum and C. bougheyanum extracts, respectively, demonstrating potent OH scavenging action. Medicinal plants analyzed may have shown antioxidant action due to the presence of phenolic components including phenolic acids and flavonoids. These phenolic compounds are effective antioxidants because of the hydrogen-donating abilities of the hydroxyls in their phenolic groups [12]. In addition, the metal ions that contribute to ROS generation may be chelated by phenolic compounds [13]. Similarities exist between our findings and those of Ngbolua et al. [24], who also found that A. manniana has antioxidant properties. It was also found by Tsafack et al. [17] that T. mauritianum has antioxidant properties.

6. CONCLUSION

The findings of this research showed that the medicinal plants examined have antibacterial and antifungal capability against drug-resistant infections. These medicinal herbs also have the potential to be utilized as an organic antioxidant supplement. The mechanism of action and potential lead molecules for the development of novel medications may be determined if the bioactive chemicals in these plant extracts were further purified and isolated.

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